PHOSPHATASES IN NORMAL AND REORGANIZING STENTORS

PAUL B. WEISZ

Arnold Biological Laboratory, Brown University, Providence, Rhode Island

The histochemical demonstration of phosphatase activity in *Stentor coeruleus*, although desirable in its own right, was undertaken primarily to determine to what extent such a study might amplify data obtained earlier (Weisz, 1949) with regard to metabolism and differentiation during normal and reorganizational stages in the life cycle of Stentor. Enzyme activity was studied in the normal, vegetative animal to provide a frame of reference, and this is compared with analogous data on starvation, regeneration, physiological reorganization, and vegetative division.

Experimental

In Stentor, phosphatases cannot be demonstrated with the usual "alkaline" techniques (e.g., Lillie, 1948). Air-dried (cf. below) or acetone-fixed test slides always yield precipitates in the same regions and in the same intensity as control slides prepared by omitting incubation or by substituting a calcium nitrate incubation for the treatment with substrate. Acid phosphatase activity, on the other hand, can be visualized.

The technique was adapted from Gomori's (1941) original method to demonstrate acid phosphatase activity. Stentors of appropriate stages were put on slides with a minimum of water, and after the organisms had been oriented, all excess water was drained off and the preparations were dried in a gentle air current from a fan. This method simultaneously flattens the animal to section thinness and fastens it to the glass. The preparations were then treated directly with the substrate.

In initial exploratory tests the usual method of fixation was tried by dropping living Stentors into chilled acetone before treating them with the substrate. It was found, however, that with this procedure much of the subsequent impregnation potential is lost, possibly due to partial enzyme inactivation by acetone (Stafford and Atkinson, 1948), possibly also due to a leaching out of some of the enzyme in the washing process (Barthelmez and Bensley, 1947). Air-drying on the other hand gave maximal and consistent results.

Of a number of substrates tried, sodium glycerophosphate was found to give the best results (cf. also Gomori, 1949), and this substrate, buffered to pH 4.7, and allowed to act for 15 to 20 hours at 25° C., was consequently used routinely. Post-incubation treatment followed the sulfide technique as outlined by Gomori. Control tests were carried out both by omitting incubation, and by poisoning the substrate with sodium fluoride (M/1000).

Recent work has raised some doubt whether the precipitates obtained by this method represent correct visualizations of phosphatase, and whether the loci of the precipitates correspond precisely to the *in vivo* sites of enzyme activity. Non-enzymatic impregnation of certain tissues by lead salts is known to occur (Lassek,

1947), especially during long incubation. The extent of this error can be estimated, however, by running controls in poisoned substrates. Inasmuch as in Stentor such control preparations do not reveal any precipitate, non-enzymatic impregnation probably does not occur to any appreciable extent under the present conditions of testing; the lead sulfide deposits obtained in the experimental material may thus be regarded presumptively as visualizations of enzyme activity. Nevertheless, in view of the possibility of enzyme shifting during the testing procedure (Barthelmez and Bensley, 1947), caution is warranted in interpreting the results, both with respect to the specificity and the localization of the reaction.

Examination of about 50 Stentors has shown that lead deposits are always found in a number of definite, circumscribed regions. In the ectoplasm, the deposits are centered in the basal granules of the body cilia and the membranelles. This gives the impression that the entire gullet and the peristome band are heavily impregnated, and that the longitudinal rows of body cilia are underscored with dark brown deposits. In the endoplasm, precipitates are particularly constant and abundant around the macronuclear nodes, but no deposits are observed within the nodes themselves. Heavy deposits are also found on the surface and probably within the endoplasmic vacuolar fat reserves (cf. Weisz, 1949), as well as in the immediate vicinity of the gastrioles. (In contrast, preparations fixed in acetone before incubation reveal only light deposits in the circumnuclear site, and no other part of the organism is impregnated.)

In starvation, a gradual decrease in the phosphatase reaction becomes manifest. Deposits in the fat vacuoles and near the gastrioles disappear first. By the time the oral area is about to be resorbed, only the regions around the macronuclei, and the basal granules of the membranelles, still yield a faint reaction (at this stage the preparations resemble those of normal animals which had been fixed in acetone). After the degeneration of the oral area even the circumnuclear activity soon disappears, and no part of the animal reveals any lead sulfide deposits. The data for physiological reorganization and vegetative division are rather parallel, and may be discussed together. The normal sites of activity largely persist unchanged throughout both types of reorganization. The point of interest centers around the regions in which new peristome bands are differentiated, anteriorly in physiological reorganization, and at mid-body in division (cf. Weisz, 1949). In every case, as in normal membranelles, newly differentiated membranelles show a high degree of activity in their basal granules. Such activity, however, can never be observed before the membranelles themselves have formed and are functional. In areas adjacent to newly formed peristome sections, i.e., in areas in which new membranelles will appear within a short time, activity is not yet evident.

Tests carried out on regenerating posterior fragments afford another opportunity to check on this point. Since the time at which new membranelles appear in a Stentor fragment is known (Weisz, 1948), it is possible to test for phosphatase before as well as after peristome new-formation. Such paired tests can be carried out on fragments obtained from the same animal. This was done, with results as above: presumptive sites of newly differentiating membranelles do not reveal any deposits; the latter become manifest only when the membranelles themselves can first be seen in an active state.

Apart from these differences in the presumptive oral area, regenerating frag-

ments do not differ from normal intact animals in the extent and the localization of the sulfide deposits.

If the deposits may indeed be regarded as visualizations of acid phosphatase activity, these observations tend to throw some light on the function of the enzymes in the basal granules of the membranelles and the body cilia, even if only in a negative sense: if the enzyme were to appear just prior to membranelle formation, a role concerned with the mechanics of ciliary differentiation and structural maintenance might be tentatively ascribed to the enzyme. Since this, however, is not the case, the enzyme may possibly be involved in the energetics of ciliary motion.

In summary, the results tend to show that phosphatases in Stentor are fairly consistently present at definite loci of the cytosome, and that reorganization processes, unless they lead to the death of an animal or a fragment, are not correlated with significant changes in enzyme distribution. Newly differentiating organelles manifest characteristic enzymatic activity in parallel with morphological differentiation as such. The presence of phosphatases at circumnuclear sites may be significant in view of evidence (Weisz, 1949) that the macronuclear nodes discharge secretions (possibly phosphate-containing nucleic acid derivatives) into the endoplasm.

Summary

Phosphatase activity is studied in *Stentor coeruleus* by means of histochemical methods. "Alkaline" procedures are negative. Acid phosphatase may be consistently demonstrated in normal Stentors around the macronuclei, in the basal granules of the membranelles and the body cilia, in the endoplasmic fat vacuoles, and around the gastrioles. During starvation a gradual decrease in intensity and distribution of enzyme activity is observed, while in regeneration, physiological reorganization, and in vegetative division, activity remains unaltered in comparison to the normal animal. Presumptive evidence is obtained indicating that acid phosphatase in the basal granules is not primarily a factor in ciliary differentiation.

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